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DEMONSTRATION OF THE EXTENT OF DROUGHT RESISTANCE IN WINTER WHEAT VARIETIES, AND STUDY OF THE PROLINE ACCUMULATION ABILITY OF 25 CULTIVATED SPECIES

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Abstract

The drought tolerance of bred varieties of one species was found to be directly proportional to the extent of proline accumulation in the isolated leaves. The water deficiency of leaves isolated at flowering time in six wheat varieties was increased up to the lethal level by means of the live-wilting method during exposure to light for 3 days. This means an identical "internal" water deficiency.

The highest proline concentrations were found in "GK Szőke" and "Jubulejnaja 50", i. e. 36% and 33% increases in proline content, respectively. Proline tests were also carried out on the isolated spikes. The lowest proline was found in "GK Örzse" and the highest proline increase in the same two varieties as found for the leaves. The increase in proline content was higher in the spikes than in the leaves, i. e. 95% and 88%, respectively. There was a heavy drought after flowering during field trials in the study year. Of the five middle-ripening varieties, "GK Szőke" produced the highest yield, as was expected on the basis of the proline tests.

Key words: Free proline, live-wilting, isolated leaves, lethal water deficiency.

Introduction

It is known that the isolated leaves of mesophyte soft-stemmed plants accumulate a huge amount of free proline in response to a severe water deficiency with optimal lighting (DASHEK and HARWOOD, 1974; TYMMS and GAFF, 1979; HUBAC and SILVA, 1980; LEVITT, 1980; PÁLFI and PINTÉR, 1980; PALEG and ASPINALL, 1981; GULYÁS and PÁLFI, 1986). It has also been reported that in the case of drought and an extensive water deficiency of the leaves, the high proline concentration has a protective effect on the plants (BLUM and EBERCON, 1976; DASHEK and HILLS, 1981; SIMINOVITCH and CLOUTIER, 1981; PÁLFI et al., 1983; BISWAS and CHOUDHURI, 1984; JOYCE et al., 1984; VAN SWAAIJ et al., 1985).

It has been concluted from the high proline concentration that those species accumulating more proline under conditions of equal water deficiency display a better drought tolerance. Our results, however, have not supported these findings. Examinations of the water deficiency of isolated leaves and shoots of 14 cultivated species revealed that the drought tolerance of a particular species is not connected with its proline accumulation ability. This conclusion is supported by the experimental results of WALDER and TEAVE (1974) in soy bean and sorghum, and of PATEL and VORA (1985) in wheat, *Plantago ovata, Papaver somniferum* and *Brassica juncea,* and by the practical results of plant breeding: among the varieties of one species, those have a better drought tolerance which are capable of accumulating proline induced by a gradually increasing water deficiency (PÁLFI at al., 1978, 1983; PÁLFI and GULYÁS, 1986).

Consequently, the proline test can be used to determine the drought tolerance of the varieties of one species. Comparisons of sunflower varieties (SAVINIVASA, 1977), ground nut varieties (SASHIDHAR et al., 1977) and maize varieties (PINTÉR et al., 1979) have shown that the level of proline accumulation connected with the drought tolerance is a heritable trait. This was confirmed by the results of BLUM and EBERCON (1976), MALI and MEHTA (1977), LEVITT (1980) and PALEG and ASPINALL (1981). VAN DE DIJK (1981) found that a given level of "external" water deficiency can cause different levels of "internal" water deficiency, which is actually the main difference between wheat varieties as concerns drought resistance. Accordingly, in determinations of the drought tolerance of different varieties, the "internal" water deficiency of the shoots and leaves must be equal. In the present study, a gradually increasing water deficiency was induced in leaves isolated at flowering time in 6 wheat varieties, with the aim of creating an identical level of "internal" water deficiency. This was followed by determination of the proline content of the water deficient leaves. Proline analysis was also carried out in the spikes of the same varieties following establishment of the constant water deficiency level. The average lenght of the upper two leaves of the shoots was measured and compared with the extent of proline accumulation. In water deficient shoots and isolated leaves of several cultivated varieties, the proline accumulation ability and the free total amino acid content were studied.

Materials and Methods

The 6 wheat varieties used in this study were bred and maintained in the Cereal Research Institute at Szeged. The names of the varieties can be seen in Table 1. Since the water deficiency is most acute at flowering and the following developmental stage in Hungary, the leaves were removed when the first and partly empty anthers appeared in the middle of the ear. This means that samples were taken from each variety at the same developmental stage. The water content of the soil was still optimal at flowering, since 45 mm of precipitation had fallen during the previous 4 weeks. In order to provoke a water deficiency, the two upper leaves of the 24 shoots of each variety were cut off.

The average lenght of the leaves were measured, and the leaves were then laid out in two groups in each variety separately. The groups of leaves were fixed adhesive tape on a plate covered by filter paper. The live-wilting was carried out under the following conditions; 26—28 °C air temperature, with 90% relative humidity for 60 hours and with 5000 lux lighting (in a phytotron chamber). During the last 12 hours of the live-wilting, the humidity was reduced to 60% in order to ensure the lethal water deficiency. The induction of water deficiency during 3 days is called live-wilting. This provoked water deficiency ensures that the levels of "internal" water deficiency of the varieties are equal.

Following live-wilting, the leaves were cut into small pieces, dried at 90 °C and then ground to powder. The leaf powder was dried further at 105 °C, and finally sealed hermetically in bottles.

The spikes of the same shoot were also cut off, subjected to the artifical live-wilting procedure for 3 days, then dried and ground prior to determination of the proline content. The lethal water deficiency provoked by the new method of live-wilting of the isolated leaves, living for at least 60 hours, led to the accumulation of a high proline concentration. The proline content of the leaves varied with the varieties. Those varieties capable of accumulating more proline at a given water deficiency level have a better drought resistance (SINGH et al., 1972; BRITIKOV, 1975; BLUM and EBERCON, 1976; MALI and MEHTA, 1977; SRINIVASA, 1977; LEVITT, 1980).

The proline analysis of the leaf powder was carried out according to Paleg and Aspinall (1981) and the total amino acid content was measured according to Rosen (1957).

Results and Discussion

The shoots separated from the root system and the isolated leaves lost a considerable amount of water immediately after the cutting and within the next 24 hours (if the vapour content of the air was saturated). Following this, the leaves became self regulated, resulting in an increase in the proline accumulation which slowed down further loss of water. The accumulated proline contents of the isolated leaves of wheat varieties provoked by live-wilting can be seen in Table 1.

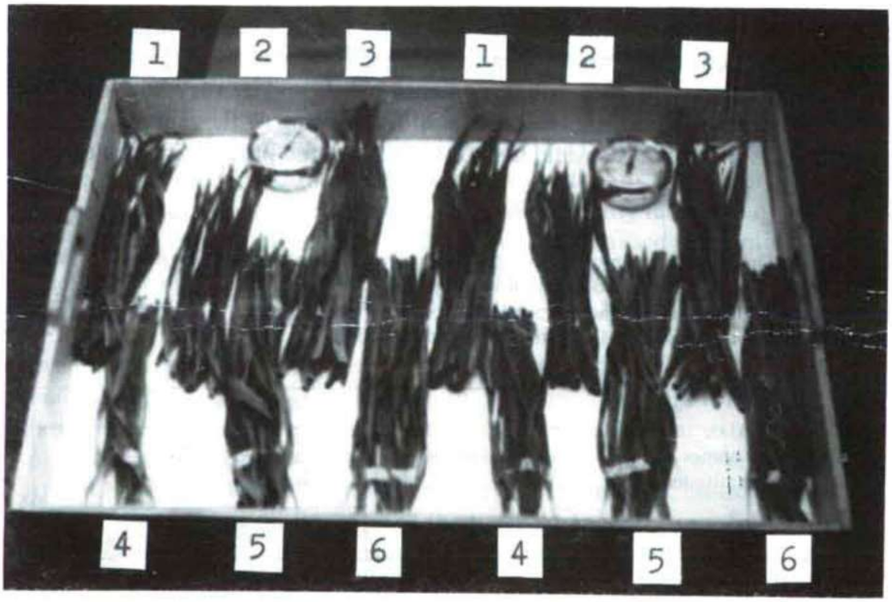

Fig. 1. Provokation of water deficiency in isolated wheat leaves by means of live-wilting for 3 days. 40 isolated leaves were laid out and fixed in two groups for each of the 6 varieties in order to ensure even lighting. The light intensity was 5000 lux, the air temperature was 26—28 °C, and the relative humidity of the air during the first two and a half days was 90%, and during the last 12 hours was 65%.

Varieties:

1 = Bucsányi 20 2 = GK István 3 = GK Szőke 4= Jubilejnaja 50 5 = GK Bence 6 = GK Őrzse

Table 1 shows that the isolated leaves accumulated different amounts of proline as a result of live-wilting or lethal water deficiency. The proline contents of two wheat varieties were outstanding: "GK Szőke" and "Jubilejnaja 50", with 3,17% and 3,10% total proline content, respectively. This means a 36% and 33% increase in proline content, respectively relative to the lowest level, in "GK Örzse". The proline contents of "Bucsányi 20" and "GK István" increased by 9,4% and 12,9%, respectively.

The application of live-wilting to provoke the lethal water deficiency of isolated leaves may solve the debate between those who elaborated the proline test and have successfully used it (SINGH et al., 1972; BLUM and EBERCON, 1976; MALI and MEHTA, 1977; PALEG and ASPINALL, 1981; SIMINOVITCH and CLOUTIER, 1981; VAN DE DIJK, 1981; VAN SVAAIJ et al., 1985), and those who are against it (GUPTA and SHEOVAN, 1979; HANSON et al., 1977, 1979; ILAHI and DÖRFFLING, 1982).

The latter group induced a constant "external" water deficiency level, but the "internal" water deficiency level was different in the living leaves (this is the point when the varieties differ from each other in drought tolerance).

The presented live-wilting method ensures that the leaf samples taken at flowering time have a constant "internal" water deficiency level, which is the lethal level. The data in Table 1 suggest that the varieties with medium leaf lenght are advantageous from the aspect of resistance.

The accumulated proline contents of isolated spikes in response to live-wilting or lethal water deficiency can be seen in Table 2.

Table 2 shows that the proline contents in the spikes vary with the varieties more significantly than during the process of live-wilting of the leaves, ranging from 0,61% to 1,19% proline. It should also be noted that the proline accumulation of the

Table 1. The concentration of synthesized proline resulting from live-wilting for 3 days and lethal water deficiency of wheat leaves isolated at flowering time. The concentration of proline is expressed as a percentage of the dry material. The average lenght of the upper two leaves is also reported. The proline content is also given as a percentage of the lowest proline content.

The names of wheatstrains	Length of leaf in cm	Concentration of proline	
		Dry substance	Minimal proline
		in per cent	
1. Bucsányi 20	29	2.55	109.4
2. GK István	27	2.63	112.9
3. GK Szőke	29	3.17	136.1
4. Jubilejnaja 50	27	3.10	133.0
5. GK Bence	30	2.46	106.0
6. GK Örzse	30	2.33	100.0

Table 2. Accumulated proline concentration of live-wilted leaves as a result of a lethal water deficiency. The live-wilting was carried out for 3 days at flowering time.

The names of wheatstrains	Concentration of proline	
	Dry substance	Minimal proline
	in per cent	
1. Bucsányi 20	0.94	154.1
2. GK István	0.65	106.5
3. GK Szőke	1.15	188.5
4. Jubilejnaja 50	1.19	195.1
5. GK Bence	0.86	141.0
6. GK Örzse	0.61	100.0

leaves is 3—4 times higher than that of the spikes. When the proline contents in the spikes of the varieties were compared, the lowest one and the two highest turned out to be the same as in the leaves. The lowest proline level was detected in "GK Örzse" (100%), while "GK Szőke" and "Jubilejnaja 50" showed the highest increases in proline: 188% and 195%, respectively.

The drought resistance data obtained by using the live-wilting of isolated leaves were supported by the practical results. HARMATY (1988) carried out field trials in the same year in lime meadow soil and on sandy soil with bad water management.

Because of the lack of precipitation and the high daily temperature (35—40 °C), a drought followed the flowering. This hot weather caused the grains to become stunted and the yield decreased. On both types of soil, from the middle-ripening group "GK Szőke" produced the highest yield. This variety was found to be the most drought-resistant at flowering time in our trials too. "Jubilejnaja 50" displayed considerable drought resistance and was the fifth out of 10 in yield production in meadow soil, and the third out of 12 in sandy soil. It is known that this standard variety is capable of producing an average yield under extreme conditions. The proline accumulation capacities of different species ar shown in Table 3.

It can be seen in Table 3 that the 25 species examined in this experiment can be devided into two groups on the basis of the proline concentration. In the first group, the proline accumulation in the leaves is extremely high, between 1,0% and 4%. In the second group, the proline remains well under 1,0%, although in these species the proline content also increased by 300—500% as compared with the control supplied with adequate water (PÁLFI et al., 1983).

It should be noted that we have studied the proline concentrations of isolated leaves of varieties storing proline in smaller quantity for 20 years (PÁLFI, 1968; PÁLFI et al., 1974, 1975, 1978, etc.). These results strongly support the data in Table

Table 3. Proline accumulation ability of various species, resulting from the lethal water deficiency of the isolated leaves. As a result of live-wilting for 3 days, the proline and total amino acid concentrations increased in mono- and dicotyledonous species belonging in one family (the species are separated by lines within the families). The samples were taken at flowering time. The live-wilting was carried out with the whole isolated shoot in the case of species with small leaves, such as *Medicago* and *Trifolium*. The concentrations are given as percentages of the dry material.

Species	Proline	Total amino acid
	concentration, per cent	
Dicotyledon		
Medicago sativa	3.64	9.12
Trifolium repens	2.37	8.25
Pisum sativum	1.82	8.76
Lens culinaris	1.53	6.34
Phaseolus vulgaris	0.56	6.87
Solanum tuberosum	1.81	7.23
S. lycopersicum	1.64	7.82
Capsicum annuum	2.89	9.16
Nicotiana tabacum	2.57	8.06
Brassica oleracea	3.88	10.64
B. napus	2.42	8.25
Raphanus sativus	1.63	7.27
Cucurbita pepo	0.38	6.56
C. maxima	0.45	6.43
Cucumis sativus	0.42	6.89
C. melo	0.36	6.74
Helianthus annuus	1.67	7.23
Lactuca sativa	0.35	7.08
Spinacea oleracea	0.46	8.12
Beta vulgaris	0.38	7.25
Monocotyledon		
Triticum aestivum	3.14	10.16
Hordeum vulgare	2.28	8.06
Secale cereale	1.93	8.78
Zea mays	0.42	9.34
Sorgum vulgare	0.51	9.27

3, showing that these species really have a low proline accumulation ability (well under 1,0%).

The mono- and dicotyledonous 25 species belong in 7 families. It can be seen that species having an extremely high or low proline accumulation ability can belong in one family. Consequently, the proline accumulation ability induced by the lethal water deficiency of the isolated leaves is not characteristic for certain families. Although general consequences can not be drawn from these results, they can serve as a good basis for further studies of proline synthesis and oxidation and the enzymes taking part in these processes.

Table 3 shows that the total amino acid contents of the species are high, varying between 6% and 11%. This suggests that the lethal water deficiency substantially increases not only the concentration of proline, but also those of other amino acids. At optimal water supply, the total amino acid content usually varies between 1,0% and 2,0%. It should also be considered that the proline concentration induced by the dry soil in intact plants grown in the field is substantially lower (by about 0,3—0,5%) than that of the isolated leaves (PÁLFI et al., 1974, 1975, 1978).

The water supply in the isolated leaves is cut off, while in the intact plants, even in the case of an extreme drought, the roots penetrating deeply into the soil supply the plant with a moderate amount of water. This is why a lethal water deficiency very rarely occurs in crop plants, though the yield can be seriously decreased by drought. The present proline data and the results of the proline test relate exclusively to isolated leaves. This is in good agreement with other studies which used isolated leaves or segments of leaves of plants grown in culture dishes (SINGH et al., 1972; BLUM and EBERCON, 1976; MALI and MEHTA, 1977; GUPTA and SHEOVAN, 1979; HANSON et al., 1979; SIMINOVITCH and CLOUTIER, 1981; THAKUR et al., 1988; etc).

These authors have already reported on the role and advantages of proline as compared with other amino acids regarding drought resistance. We have also described this in detail (PÁLFI et al., 1975, 1975, 1983).

It should also be taken into consideration that the proline accumulation ability is an evolutionary, heritable trait (BLUM and EBERCON, 1976; LEVITT, 1980; PALEG and ASPINALL, 1981; VAN SWAAIJ et al., 1985). This trait of a particular species or bred variety could presumably be transferred by means of traditional crossing or genetic manipulation to other high-yielding varieties which do not have sufficient drought resistance (WYN JONES and CORHAM, 1986). Throught the crossing of related inbred maize lines, this has already been carried out successfully in part (PINTÉR et al., 1981).

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